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Screening of Particular Food-borne Pathogens in Raw Buffalos' milk and some Popular Artisanal Egyptian Dairy Products

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Abstract

Although their nutritive value and popularity, milk and dairy products frequently serve as a vehicle for the various pathogens of both human and animal origins. A wide variety of artisanal dairy products are produced in rural areas and villages of Egypt and due to lack of strict hygienic measures and lack of thermal treatment, these products usually harbor a variety of spoilage and possibly food-poisoning microorganisms. The current study aimed to investigate the contamination incidence of raw milk, Kariesh cheese and artisanal Yoghurt (Zabady) by *Staphylococci*, *E. coli* and *Bacillus cereus*. Overall, 75 samples of raw milk (100.00%) were contaminated by *Staphylococci*, 54 samples of karish cheese (72.00 %) contained *E. coli*, meanwhile, 45 samples of Zabady (60.00 %) yielded *Bacillus cereus*. Additionally, 29 out of 34 *E. coli* strains recovered from examined samples, were successfully serotyped with correspondence to 9 different serogroups, meanwhile 43 strains from different samples were untypable by available antisera. 80 out of all 162 *E. coli* isolates (49.38 %) carried haemolytic activity feature, which reflect a great threat towards consumers. Furthermore, the phenotypic AR of *S. aureus* and *E. coli* isolates was checked against eleven selected antibiotics. A remarkable variation in phenotypic AR was noticed among strains. Our results denote the high incidence of health hazards in raw milk and its products, and the existence of AR *S. aureus* and *E. coli* strains isolated from milk and dairy products in rural areas, which could cause human illnesses that are difficult to be treated.

KEYWORDS
Food-borne Pathogens, Buffalo, Milk, *E. coli*, *S. aureus*

INTRODUCTION

Milk and different processed dairy products constitute an important nutritional category of human food. However, they also can serve as a good medium for the growth of many undesirable microorganisms of spoilage or even pathogenic characters.

Among typical artisanal dairy products in Egypt, Karish, cheese and artisanal yoghurt (Zabady) are extensively manufactured in the rural areas and are desired by a wide variety of consumers. Karish cheese is a type of acid-coagulated cheeses made from defatted or skim cow or buffalo's milk in a special earthenware pots on which the partly skimmed milk sours and clots (Korish and Abd Elhamid, 2012). Later, the curd is poured onto a special mat which is tied and hung with its contents to allow the drainage of the whey over a period of 2-3 days until the desired texture of the cheese is obtained. Finally, the cheese is salted and cut into suitable pieces to be left for a few hours in the mat till no more whey drains out. Like Domiati type, Karish cheese can be ready to be consumed as a "fresh Karish" within 1-2 weeks or can be pickled in brine earthenware pots; the so called "Mish cheese" to maintain a valid shelf-life for up to a year (Abou-Donia, 2008; Baraheem *et al*., 2007). This cheese is one of the most popular types of soft cheese consumed in Egypt, especially in the countryside and among athletics owing to its high protein content (30%), low fat and lowered price (Abou-Donia, 2008; Ahmed *et al*., 2005; Alnakip, 2009; Korish and Abd Elhamid, 2012). The quantity of Karish cheese produced in Egypt, is unknown, however, it is believed that about 50% of the total milk produced is utilized for its manufacturing (Baraheem *et al*., 2007). On the other hand, artisanal yoghurt (Zabady) is manufactured by various producers via inoculation of previously boiled milk with yoghurt either from commercial factories or yoghurt produced locally from the previous days. Thus, it differs from commercial yoghurt in the purity of starter as its manufacture does not include the addition of starters in their pure form. Such method of production of artisanal yoghurt does not guarantee the purity and the safety of starter as additive fermentation inoculum. As inferred from production process, production facilities are quite primitive, the incubation conditions of those products are relatively uncontrolled accompanied with lack of strict hygienic measures and secure thermal treatment.

The genus *Staphylococcus* represents serious challenges for both veterinary and public health sectors. Among members of this genus, *Staphylococcus aureus* is considered as one of the leading causes of food-intoxication due to production of a wide range of heat-stable enterotoxins, as well as toxic shock syndrome toxin type-1 (TSST-1). Furthermore, *S. aureus* is a major cause of several disorders such as bone and joint infections, and septicemia (Juhász-Kaszanyitzky *et al*., 2007, Kamal *et al*., 2018). On the other hand, coagulase-negative *Staphylococci* (CoNS) also possess several threats towards human, and animals, and being documented as most isolated minor mastitis agents: particularly responsible for subacute and chronic patterns.

Escherichia coli are common food-borne bacteria frequently

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associated with milk and dairy products and strongly incriminated in food-poisoning outbreaks. *E. coli* are frequent component of the gut microbiome of most warm-blooded organisms including humans, thus their presence in foods indicates mainly faecal pollution. In dairy sector, the occurrence of *E. coli* contamination seems to be greater in raw and non-thermally treated dairy products or exists within post-processing of thermally-treated dairy products (Bell and Kyriakides, 1998; Bielaszewska *et al*., 2000); since the bacterium usually does not survive several preservation processes (Law, 2000).

There are at least 200 serotypes of *E. coli* that are capable of producing Shiga toxins (STs), and are known as STs producing *E. coli* (STEC) (Nataro and Kaper, 1998; Pradel *et al*., 2000, Hamdy *et al*., 2023), but only a few are related to severe human diseases and most infections are caused by O157:H7 serotype (Boyce *et al*., 1995; Law, 2000). Other major STEC serogroups associated with pathogenicity include O111, O26, O6, OX3, O91 and O103; which have been identified in bovine feces and in food samples (Paton and Paton, 1998; Pradel *et al*., 2000; Samadpour *et al*., 1994). STEC, particularly O157:H7 serotype was emerged as important food-borne pathogens associated with various human diseases, including watery diarrhea, hemorrhagic colitis (HC), hemolytic uremic syndrome (HUS) and thrombotic thrombocytopenic purpura (TTP) in humans (Deisingh and Thompson, 2004; Fitzpatrick, 1999; Law, 2000; Paton and Paton, 1998).

Bacillus cereus remains of a great public health concern due to several issues. This species can survive several heat treatment processes, and in addition, some strains are psychro-tolerant, thus it can limit the keeping quality of both pasteurized and refrigerated dairy products. Furthermore, *B. cereus* represents several crucial risks for both human beings; since it is implicated in food poisoning (FP) (Zhou *et al*., 2008; Alnakip, 2014; Caamaño-Antelo *et al*., 2015). Consumption of milk and dairy products has been reported to be strongly connected to *B. cereus* FP outbreaks (Salah *et al*., 2016). *B. cereus* belongs to the Hazard group 2 organisms as defined in the European legislation (European Commission Council Directive 93/88/EEC). FP caused by such bacterium occurs in two types of illness: the emetic and diarrheal syndromes (Logan, 2012). It has been reported that *B. cereus* is responsible for nearly about 5% of food-borne outbreaks in the England, Netherlands, France, and USA (Rosenquist *et al*., 2005).

Antimicrobial resistance (AR) has developed as one of the major urgent threats to public health causing serious issues to successful prevention and treatment of persistent diseases. Misusing and overusing different antibacterial agents in the health care setting as well as in the dairy industry are considered among the major reasons behind the emergence of AR. The threat of AR is of particular importance in the category of antibiotic resistance in bacteria.

Because of the great distribution of raw buffalo`s milk among farmers and popular consumption of artisanal karish cheese and Zabady; that are processed from raw buffalo`s milk, the current study aimed to investigate the contamination incidence of aforementioned foods by *Staphylococci*, *E. coli* and *Bacillus cereus*. Additionally, the antimicrobial resistance of isolates was determined.

MATERIALS AND METHODS

Collection of samples

A total of 75 samples of raw buffalos' milk, artisanal Kariesh cheese and artisanal Yoghurt (Zabady) made from buffalos' milk (25 samples of each) were aseptically sampled from AlSharkia Governorate markets, Egypt. All samples were aseptically collected in sterile containers and transported rapidly in a 4°C-vehicle-mounted refrigerator to the Laboratory of Food Control Department, Faculty of Veterinary Medicine, Zagazig University to be investigated microbiologically within few hours according to accredited protocols.

Preparation of samples for Microbiological investigations

Samples were adequately prepared and serially diluted according to the guidelines described by IDF (1992); Wehr and Frank (2004). Accordingly, 1 ml of each raw milk samples was mixed to 9 ml sterile distilled water and undergone serial dilution to be ready for culture. Meanwhile, for Kariesh cheese and yoghurt, 11 grams of each sample were homogenized and thoroughly mashed in 99 ml of 0.1% sterile peptone water (40°C) inside a clean sterile mortar under sterile condition. After the mixture become homogenous, 1 ml from the cheese solution is used for preparation of decimal dilution as previously described for milk samples.

Microbiological Examination

Isolation and Identification of *Staphylococci* isolates

Staphylococci were isolated from previously prepared decimal serial dilutions and plated on Baird Parker agar (Oxoid). Characteristic colonies (Black with and without opaque halo) were picked up, and each one underwent purification process by re-streaking for two successive times on fresh Baird Parker agar medium plates. After the second purification step, all isolates were coded and kept at -20°C, thus the pure strains become adapted for deep freezing (-20°C), for additional investigations when required.

Primary characterization of *S. aureus* was dependent on colony characteristics, Gram staining and biochemical characteristics (Oxidase, Catalase, mannitol fermentation, Tube coagulase test and Deoxyribonuclease (D-Nase activity) tests). For performing coagulase test (CT), each suspected colony was inoculated into peptone water (Merck, Germany) at 37°C for 24 hours. Later, 0.5 mL of each tube was transferred aseptically to sterile tube contains 0.5 mL of reconstituted rabbit plasma (STAPH-ASE) (Biomérieux, France) followed by vortexing. The tubes were then incubated at 37°C for 1-3 hours to assess the coagulation activity. Additional incubation for another 24 hours was done for negative reactions. The assessment of coagulation reaction was observed by gently inclining each tube to avoid breaking of any developed clot. A negative control tube was done in parallel.

Isolation, Identification and serotyping of *E. coli* isolates

One ml from each sample was inoculated in a tube of sterile buffered peptone water (BPW) and incubated aerobically at 37°C for 24 h. Later, one ml from the incubated BPW was transferred to 10 ml MacConkey broth and incubated at 37°C for 24 h. Approximately 0.1 mL from the incubated broth was streaked on Eosin Methylene Blue (EMB) agar and incubated at 37°C for 24 h. Colonies with typical morphological characteristics (green-metallic sheen colonies) were picked up from each plate and further purified by re-streaking two successive times on fresh EMB plates. Primary characterization of *E. coli* was dependent on Gram staining and biochemical characteristics. All suspected *E. coli* isolates were maintained as frozen cultures in Tryptone Soya broth

(Oxoid, UK) and 50% glycerol at -80°C.

Primary Identification of *E. coli* was dependent on Biochemical characteristics. The suspected isolates were confirmed by biochemical tests as Indol production test, Voges-Proskauer test, Methyl Red Test, Citrate utilization test and Production of gas from lactose test. *E. coli* isolates were positive for Indol, Methyl red tests and negative for Vogues Proskauer and citrate utilization tests.

Serological typing of *E. coli* isolates: Serotyping of *E. coli* was performed by slide agglutination test using polyvalent and monovalent antisera according to the instructions of the manufacturer (Bio-Rad Laboratories, Marnes-la-Coquette, France).

Detection of hemolytic activity: Production of Ehly was assayed acc. to method by Beutin *et al*. (1989) and Beutin *et al*., (1993). Different *E. coli* isolates were incubated in MacConkey broth. A loopful from each broth culture was streaked onto blood agar medium supplemented with 5% defibrinated sheep blood. The plates were incubated at 37°C for 24 hours. ß-Hemolysis was defined as a zone of complete erythrocyte lysis surrounding a bacterial colony.

PCR-based Detection of stx genes in E. coli isolates

Total genomic DNA was extracted from overnight cultures of E .coli isolates as previously described by (Quintela-Baluja *et al*., 2013). The bacterial cells were lysed by the addition of 180 µL of lysis solution (Sigma-Aldrich) after incubation for 2 h at 37ºC. Total genomic DNA was extracted and purified using the DNeasy Tissue Mini Kit (Qiagen) and kept stored in a -20°C freezer. The *stx* genes were amplified by PCR using primer pairs illustrated in Table (A) as previously described by (Paton and Paton, 1998; Kaur, 2015). All the PCR assays performed using a "My Cycler" Thermal Cycler (Bio-Rad Laboratories, USA). PCR assay consisted of 35 cycles, each consisting of 1 min of denaturation at 95°C; 2 min of annealing at 65°C for the first 10 cycles, decrementing to 60°C by cycle 15; and 1.5 min of elongation at 72°C, incrementing to 2.5 min from cycles 25 to 35. The results of PCR visualized after agarose gel electrophoresis under UV trans-illuminator.

Isolation and Enumeration of B. cereus

Primary enrichment was done by adding 1.0 ml of each sample to 9.0 ml of nutrient broth (Oxoid, UK) followed by incubation at 34°C for 24 hrs. All presumptive positive (Turbid) enrichment cultures were further streaked on MYP agar plates (Oxoid, UK), followed by incubation of the plates at 30°C for 18-24 h (Banykó and Vyletělová, 2009; Němečková *et al*., 2011). The number of *B. cereus*-like organisms was estimated according to standard procedure (Rosenquist *et al*., 2005).

The suspected growing colonies on MYP agar plates (Large, grayish to greenish, circular colonies with a β-haemolytic pattern and ground glass appearance or cloudy halo due to lecithinase production) were considered as presumptive *B. cereus* isolates, and picked for further streaking on trypticase soy-sheep blood agar plates (Oxoid, UK) to observe haemolytic patterns after incubation at 35°C for 24 h (Salah *et al*., 2016). Large, grayish to greenish, circular colonies with a β-haemolytic pattern and ground glass appearance on blood agar plates were preliminarily proposed as *B. cereus* and subjected to further confirmatory biochemical examination acc. to standard protocols (Banykó and Vyletělová, 2009; Salah *et al*., 2016).

Antibiotic susceptibility testing

For their role in causing various diseases and microbial disorders in human, All *S. aureus* strains (36 isolate) plus third of *E. coli* (isolates (54 isolates) were tested for susceptibility to eleven antimicrobials using the disc diffusion method on Mueller-Hinton agar (Oxoid), and according to standard instructions (Clinical and Laboratory Standards Institute "CLSI", 2008). The antibiotic discs (antibiotic concentration in µg) were as follows: ampicillin (25), Amoxycillin (30), Chloramphenicol (30), Ceftriaxone sodium (15), Erythromycin (15), Gentamycin (15), Cefoperazone (15), Streptomycin (2), Tetracycline (20), cefoxitin (15), vancomycin (30) and Cefotaxime (20). Zones of growth inhibition were measured, and the interpretation of results was accomplished following CLSI guidelines, whereby intermediate results were considered resistant.

RESULTS AND DISCUSSION

Although their nutritive value and popularity, milk and dairy products frequently serve as a vehicle for the various pathogens of both human and animal origins. A wide variety of artisanal cheeses are produced in rural areas and villages of Egypt without addition of any starter as the fermentation process is mainly dependent on native wild microflora (Fahmy and Youssef, 1978; Dufour and Collin, 1995; El-Soda *et al*., 2003; El-Baradei *et al*., 2007; El-Baradei *et al*., 2008; Alnakip, 2009). Due to lack of strict hygienic measures and lack of thermal treatment, these products usually harbor a variety of spoilage and possibly food-poisoning microorganisms.

Seventy-five samples of raw buffalo`s milk, Kariesh cheese and Zabady (25 of each) were bacteriologically tested for the contamination by *Staphylococci*, *E. coli* and *B. cereus*. Overall, 75 samples of raw milk (100.00%) were contaminated by *Staphylococci*, 54 samples of karish cheese (72.00 %) contained *E. coli*, meanwhile, 45 samples of Zabady (60.00 %) yielded *Bacillus*-like growth (Table 1). As shown in Table 2, the discrimination of *Staphylococci* isolates from different sources is illustrated based on biochemical characteristics. All samples were contaminated by *Staphylococci*, which reflect the serious neglected sanitary production procedures among artisanal producers during production and distribution cycle and reflect the great impact of devoid of production processes of such products from any thermal treatment, thus raw milk contaminating microflora continued to the processed products. For *E. coli*. The incidence of *E. coli* was the highest in raw milk (53.85%) followed in order by karish cheese (72.00%) and artisanal Zabady (60.00 %). Like raw milk, *E. coli* was isolated from various karish cheese and Zabady samples. This is could be attributed to either incorporation of unpasteurized milk *et al*., 2015) coupled with primitive equipments used in manufacture

Table A. PCR primers used for targeting *stx* genes.

Primer	Sequence (5′-3′)	Specificity	Amplicon size (bp)
*stx1*F *stx1*R	ATAAATCGCCATTCGTTGACTAC AGAACGCCCACTGAGATCATC	nt 454–633 of A subunit coding region of *stx*1	180
*stx2*F *stx2*R	GGCACTGTCTGAAACTGCTCC TCGCCAGTTATCTGACATTCTG	nt 603–857 of A subunit coding region of *stx*2 (including *stx*2 variants)	255

of such products and or poor sanitation during various stages of preparation, storage and distribution (Alnakip, 2009; Kumar and Prasad, 2010). Compared to our results, nearly similar values were recorded for contamination of various milk products (Maity *et al*., 2010), higher values were recorded by (Soomro *et al*., 2002), while lower incidence percentages were reported by (Öksüz *et al*., 2004; Kumar and Prasad, 2010; Zeinhom and Abdel-Latef, 2014; El-Sharoud *et al*., 2015; Alnakip *et al*., 2023). Kumar and Prasad (2010) found that contamination with *E. coli* was higher in raw milk from vendors (13.33 %) compared to that collected from dairy farms (6.67 %).

As shown in Table 2, the typing of *Staphylococci* revealed the predominance of *S. aureus* in raw milk (16 %) and Kariesh cheese (24%) samples. Meanwhile, *S. epidermidis* was the predominant staphylococcal species in Zabady samples. Additionally, based on biochemical identification: 13.33, 29.33 and 14.67 % of isolates from raw milk, Kariesh cheese and Zabady, respectively were untypable.

As shown in Table 3, 29 out of 34 *E. coli* strains recovered from examined samples, were successfully serotyped with correspondence to 9 different serogroups, meanwhile 43 strains from different samples were untypable by available antisera. In raw milk, the predominant serotype was O45:H38 (18.33 %) followed in order by O111:H4 (15%) O157:H7 (13.33 %), O91:H21 (10%) and O26:H11 (8.33 %), O91:H28 and O27:H18 (6.67 % of each) and O76:H19 (3.33 %). Various literatures showed isolation of aforementioned *E. coli* serotypes from raw milk and artisanal dairy products prepared from raw milk in various percentages. (Lye *et al*., 2013; Mansouri-Najand and Khalili, 2007; Öksüz *et al*., 2004; Picozzi *et al*., 2005; Rey *et al*., 2006). Particularly, *E. coli* O157:H7 was isolated at the rates of 0.3% (Rey *et al*., 2006), 1% (Öksüz *et al*., 2004) and 8.75 % (Lye *et al*., 2013) out of examined samples. On the other hand, in a study by Rey *et al*. (2006), 8 different serotypes such as O27:H18, O45:H38, O76:H19, O91:H28, O157:H7, ONT:H7, ONT:H9 and ONT:H21, have been identified in unpasteurized milk, fresh cheese curds and cheeses; among the serotype O27:H18; which has not been reported previously as STEC. Also, in a study by Pradel *et al*. (2000), other *E. coli* serotypes as OX3:H2 and O91:H21; that are known to associate HUS, have been isolated from cheeses. Haemolytic activity is considered as an important virulence factor for some strains of *E. coli* to overcome host defense mechanism through enterohaemolysin production which is related to release of iron into the bacterial environment and cytotoxic effect towards attacking neutrophils (Cavalieri and Snyder, 1982; Schmidt *et al*., 1995). As illustrated in Table (3), 80 out of all 162 *E. coli* isolates (49.38 %) carried this feature, which reflect a great threat towards consumers.

Several phenotypic assays were developed for the detection of antibiotic resistance such as disk diffusion test, minimum inhibitory concentration determination and automated assays, like MRSA latex agglutination test, Microscan and Vitek-2 system (Felten *et al*., 2002; Moon *et al*., 2007; Turutoglu *et al*., 2006; Feßler *et al*., 2010; Kamal *et al*., 2013; Alnakip *et al*., 2019).

The phenotypic AR of *S. aureus* and *E. coli* isolates was checked against eleven selected antibiotics (Tables 4, 5). A remarkable variation in phenotypic AR was noticed among strains. In similar studies (Sampimon *et al*., 2011), isolates showed phenotypic resistance in variable percentages to several antibiotics such as oxacillin, penicillin, ampicillin, erythromycin, tetracycline, clindamycin, ciprofloxacin, cefoxitin, and gentamycin. A wide spectrum phenotypic-AR has been described previously for methicillin-resistant *Staphylococci* of dairy origin (Sawant *et al*., 2009; Feßler *et al*., 2010; Sampimon *et al*., 2011).

The use of antibiotics in agriculture farming is mostly for ther-

Table 1. *Staphylococci*, *E. coli* and *Bacillus cereus* counts/ml in examined raw milk, Kariesh cheese and Zabady samples.

Samples	No. of samples	*Staphylococci*				*E. coli*				*Bacillus cereus*			
		Positive		*Staphylococci* count/ml		Positive		*E. coli* count/ml		Positive		*B. cereus* count/ml	
		No.	%	Min.	Max.	Mean ±S.E.M.	No.	%	Min.	Max.	Mean ±S.E.M.	No.	%	Min.	Max.	Mean ±S.E.M.
Raw milk	25	25	100	1.9×10^5	8.3×10^7	$3.9\times10^6\pm2.5\times10^6$	20	80	8.9×10^5	4.9×10^7	$4.2\times10^7\pm6.3\times10^3$	15	60	1.8×10^3	6.8×10^5	$3.4\times10^4\pm1.3\times10^2$
Kariesh cheese	25	25	100	4.5×10^5	4.6×10^8	$9.7\times10^7\pm0.33\times10^7$	19	76	7.4×10^3	6.5×10^7	$9.8\times10^5\pm3.3\times10^3$	21	84	6.2×10^3	3.4×10^5	$9.8\times10^4\pm2.9\times10^2$
Zabady	25	25	100	6.5×10^6	9.3×10^8	$7.8\times10^7\pm0.33\times10^7$	15	60	4.7×10^4	7.8×10^7	$3.6\times10^6\pm4.2\times10^4$	9	36	2.2×10^4	7.8×10^4	$3.6\times10^4\pm1.2\times10^2$

Table 2. Incidence of *Staphylococcus* spp. isolated from examined samples and their identification based on biochemical characteristics.

Isolates	Raw milk		Kariesh cheese		Zabady	
	No	%	No	%	No	%
S. aureus	12	16	18	24	6	8
S. epidermidis	8	10.67	11	14.67	17	22.67
S. simulans	7	9.33	4	5.33	4	5.33
S. xylosus	3	4	6	8	3	4
S. saprophyticus	6	8	0	0	6	8
S. capitis	7	9.33	3	4	3	4
S. chromogenes	7	9.33	3	4	6	8
S. equorum	4	5.33	0	0	2	2.67
S. haemolyticus	3	4	0	0	5	6.67
S. lentus	4	5.33	6	8	6	8
S. succinus	4	5.33	2	2.67	6	8
Unidentified	10	13.33	22	29.33	11	14.67
Total	75	100	75	100	75	100

Table 3. Serotyping of *E. coli* isolates from raw milk and dairy products and their hemolytic activity.

Serotype	Incidence of serotypes per Sample						Hemolytic activity of serotypes					
	Raw milk		Kariesh cheese		Zabady		Raw milk		Kariesh cheese		Zabady	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
O_{157}:H_7	8	13.33	3	5.26	0	0	6	23.08	3	9.38	0	0
O_{111}:H_4	9	15	5	8.77	3	6.67	3	11.54	2	6.25	0	0
O_{91}:H_{21}	6	10	9	15.79	2	4.44	3	11.54	4	12.5	1	4.55
O_{91}:H_{28}	4	6.67	4	7.02	2	4.44	2	7.69	2	6.25	0	0
O_{26}:H_{11}	5	8.33	7	12.28	3	6.67	0	0	5	15.63	2	9.09
O_{127}:H_6	0	0	6	10.53	4	8.89	0	0	6	18.75	2	9.09
O_{27}:H_{18}	4	6.67	2	3.51	4	8.89	1	3.85	1	3.13	1	4.55
O_{45}:H_{38}	11	18.33	2	3.51	4	8.89	3	11.54	0	0	2	9.09
O_{76}:H_{19}	2	3.33	5	8.77	5	11.11	1	3.85	1	3.13	3	13.64
Untyped	11	18.33	14	24.56	18	40	7	26.92	8	25	11	50
Total	60	100	57	100	45	100	26	100	32	100	22	100

Table 4. Sensitivity/ resistance of examined *S. aureus* isolates against eleven selected antimicrobials.

Antibiotic	Sensitive		Resistant	
	No. of isolates	%	No. of isolates	%
Ampicillin	12	33.33	24	66.67
Chloramphenicol	10	27.78	26	72.22
Ceftriaxone sodium	19	52.78	17	47.22
Erythromycin	22	61.11	14	38.89
Gentamycin	14	38.89	22	61.11
Cefoperazone	20	55.56	16	44.44
Streptomycin	10	27.78	26	72.22
Tetracycline	8	22. 22	28	77.78
Cefoxitin	32	88.89	4	11.11
Vancomycin	5	13.89	31	86.11
Cefotaxime	24	66.67	12	33.33

apeutic purposes, and as a preventive measure during dry cow therapy, and being used in lesser extend as growth promoters (Butaye *et al*., 2003). The overuse and misuse of antibiotics in the last two decades increased the abundance of AR bacteria, which elevated the risk of emergence of resistant zoonotic bacterial pathogens (Mevius *et al*., 2005; Haran *et al*., 2012). Furthermore, the presence of mobile genetic elements (e.g, plasmids and transposons) allowed bacteria to interchange resistance genes between different bacterial species without a consideration to phylogenetic, ecological or geographical boundaries (Mevius *et al*., 2005; Leonard and Markey, 2008). Therefore, foods of animal origin are significantly linked to the spread of potential resistance bacteria to consumers, particularly raw milk and non-thermally treated dairy products, where bacteria carrying AR genes can survive. Recently, the occurrence of AR in dairy animals has been greatly linked to mastitis as being the main reason for using an-

Table 5. Sensitivity/ resistance of examined *E. coli* isolates against eleven selected antimicrobials.

Antibiotic	Sensitive		Resistant	
	No. of isolates	%	No. of isolates	%
Ampicillin	22	40.74	32	59.26
Chloramphenicol	21	38.89	33	61.11
Ceftriaxone sodium	41	75.93	13	24.07
Erythromycin	35	64.81	19	35.19
Gentamycin	24	44.44	30	55.56
Cefoperazone	29	53.71	25	46.29
Streptomycin	22	40.74	32	59.26
Tetracycline	13	24.08	41	75.92
Cefoxitin	44	81.48	10	18.52
Vancomycin	18	33.33	36	66.67
Cefotaxime	36	66.67	18	33.33

tibiotics (Lee, 2003; Prèrea *et al*., and Fayetb, 2006). According to US Centre for Disease control and Prevention (CDC), at least 2 million people in USA were infected with antibiotic-resistant bacteria and at least 23,000 cases die each year as a direct result of these infections.

CONCLUSION

Raw milk and its products constitute a potential public health hazard. Moreover, the existence of AR bacterial strains exaggerates the issue and calls for great attention to the urgent need for decisive regulations and rules to face the increasing misuse of antibiotics in dairy herds and emphasize the need for novel natural antimicrobial therapeutic agents.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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